REMARKS

By an Office Action dated July 1, 2002 in the file of this application the Examiner rejected the application on a variety of format and prior art grounds. Based on this response, reconsideration of the merits of this patent application is respectfully requested.

In the Office Action, the Examiner persevered with the requirement for restriction. Accordingly, Claims 13-16 have been withdrawn from consideration without prejudice.

The Examiner mentioned that a reference was attached to Twisk (2000). The applicants is not sure, but suspects that that document should have been a copy of US Patent No. 5,521,071 and a copy of that document is enclosed with this submission accompanied by a PTO Form-1449.

The Examiner pointed out that the application needs a sequence listing. A sequence listing has been submitted herewith in paper and in computer readable form. Please add the paper sequence listing to this application. The computer-readable and paper sequence listings are the same.

The Examiner objected to the specification because the Brief Description of the Drawings did not specify which example the data represents. The examples are not numbered in the specification and hence no numbering would have been appropriate. The data represented in Figure 1 is described in the specification on page 8, in the paragraph beginning on line 11. The data is the output of a chromatography column. An X and XY axis scale for such a chromatography column is not required so long as the identification of critical peaks is made, and Figure 1 contains a designation of the important peaks found in the chromatography output. Accordingly, it is believed the specification is complete in this regard as filed.

The Examiner imposed rejections under 35 U.S.C. §112, first paragraph in that the claims went beyond that which was enabled by the specification in a couple of regards. First the Examiner deemed that the language reciting "a low density lipoprotein receptor" in the claims was too broad. The Examiner also suggested that the receptor be recited in such a way so as to exclude the membrane binding domain of the native protein. The applicants have responded to those rejections by making the changes above to Claims 1, 5 and 9 so as to limit the claims of the application to the low density lipoprotein receptor which does not include the membrane binding domain of the native protein. Support for this language is found in the specification at the bottom of page 3, lines 27 to 29 and on page 4, lines 2 and 3. Similarly, the Examiner has objected to the specification as being incomplete by omitting essential steps, in that the claims did not specifically recite that serum cholesterol or triglyceride levels

were lowered in the patient. Language has been added to the claims to make it clear that this occurs in the individual who has been treated. It is believed that these amendments to the claims have cured the rejections under §112, first and second paragraph, applied by the Examiner in the Office Action.

The Examiner also applied a rejection to Claims 9, 11 and 13 under §103(a) arguing that the claims of the present invention were obvious over Twisk in view of Teasdale and Jackson. The applicants note that the same rejection was not made to Claim 10 which was limited to the specific form of the LDL receptor which was truncated to exclude the membrane binding domain. It is believed that the amendments made to Claim 9 above will overcome this rejection in the same way that the prior rejection was not applied against Claim 10. Accordingly it is believed that these amendments also overcome the rejection applied by the Examiner.

Wherefore reconsideration of the merits of this patent application is respectfully requested.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

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Examiner: Celine X. Qian

Title: INHIBITION OF LIPOPROTEIN SECRETION

File No.: 960296.97290

In the Specification:

Page, 1, line 6, delete the paragraph (reading "To be determined.") in its entirety and insert therefor the following:

--The invention was made with United States government support awarded by the following agencies:

NIH HL56593

The United States has certain rights in this invention.--

Page 6, paragraph beginning line 19, please substitute the following:

--Preparation of mouse hepatocytes. Hepatocytes were isolated by liver perfusion and seeded at subconfluency in Dulbecco's Modified Eagle Medium (DMEM; GIBCO-BRL supplemented with fetal bovine serum (FBS; 10% v/v; Hyclone), insulin (20mU/ml; Novo Nordisk) and dexamethasone (25nM; Sigma). Cells were left to attach for 4 hours in an incubator at 37°C with 5% CO_2. Following a wash with DMEM, the cells were cultured overnight in DMEM supplemented with 10% FBS and 20 mU/ml insulin. On the following day, cells were transfected with an expression pAdTRACKCMV- $LDLR_{KDEL}$ for a fusion protein of the LDLR truncated receptor with the KDEL localization domain, or $LDLR_{KDEL}$, or with a control plasmid. The transfections were performed using the TransIT-Insecta transfection reagent (Mirus) following the manufacturer's protocol, except that the transfections were performed using 10 [m]μg DNA and 40 [m]μl TranIT-Insecta reagent per 2 ml supplemented with DMEM and 10% FBS in a 60 mm dish of cultured hepatocytes. Transfected hepatocytes were cultured for an additional 36-48 hour period prior to further experimentation.--

Page 7, paragraph beginning line 3, please substitute the following:

--Labeling. The hepatocytes were incubated for 1 hour in starve medium before pulse-labeling for 7.5 minutes with radioactive tracer (200 [m]μCi [^{35}S]

methionine/cysteine/60 mm dish). The dishes were washed one time with DMEM prior to addition of chase medium (DMEM supplemented with 10mM each of labeled methionine and cysteine and 0.2 mM oleic acid).--

Page 7, paragraph beginning line 8, please substitute the following:

--Immunoprecipitation. Following the radiolabeling, the media were collected and centrifuged (5 min., 1000 rpm). The resulting media were used for immunoprecipitations. Cells were rinsed three times with ice-cold PBS, scraped into PBS, and collected by centrifugation. The cell pellets were lysed in 200 [m]μl RIPA/1% SDS (150 mM NaCl; 50 mM Tris (pH 7.5); 1% Triton X-100; 0.5% deoxycholate; 1% SDS; 1mM PMSF; 1 mM orthovanadate; 10 [m]μg/ml trypsin inhibitor; 10 [m]μg/ml leupeptin). The mixture was then diluted five times to 1 ml final volume in 150 mM NaCl; 50 mM Tris (pH 7.4); 1 mM PMSF; 1 mM orthovanadate; 10 mg/ml trypsin inhibitor; 10 mg/ml leupeptin. For immunoprecipitations, both the media and the cell lysates were supplemented with 1/5 volume IMB (100 mM Tris (pH 7.4), 25 mM EDTA, 5 mg/ml BSA; 2.5% sodium deoxycholate, 2.5% Triton X-100, 0.01% sodium azide). Antibodies to apoB (polyclonal, rabbit anti-pig LDL) or albumin (polyclonal, rabbit anti-human serum albumin; Sigma) were also added. For the precipitations of albumin, IMB did not contain BSA. After an overnight incubation at 4°C, Protein A-agarose beads (Gibco-BRL) were added and the incubation continued at 4°C overnight. The antibody/ bead slurry was subsequently washed, once with PBB (10 mM phosphate buffer (pH 7.4), 1 mg/ml BSA, 0.01% sodium azide) and once with PB (PBB without BSA). Radiolabeled protein was solubilized in SDS-sample buffer (2% SDS, 20% glycerol, 50mM Tris (pH 6.8), 6 M urea, 1 mM EDTA, 20 mg/ml bromophenol blue), supplemented with 10 mM DTT and 250 mM [b]β-mercaptoethanol, and heated at 65°C for 30 minutes prior to SDS polyacrylamide gel electrophoresis (SDS-PAGE). Specific proteins were visualized by autoradiography and the amounts on unlabeled protein were determined by storage phosphor technology (PhosphorImager, Molecular Dynamics; ImageQuant version 3.3). All data was normalized to cellular protein and total TCA-precipitable radiation.--

Page 8, paragraph beginning line 11, please substitute the following:

--In a first trial, plasmids encoding either $LDLR_{KDEL}$ or [b]β-galactosidase (control) were injected into a tail vein of mice lacking a functional LDL receptor. Approximately 48 hours after the injection, the mice were fasted for 4 hours and then sacrificed. Plasma from the mice was harvested, diluted 1:1 with PBS, filtered and fractionated using a Pharmacia

Sepharose 6 column. The protein profile from that analysis is illustrated in Fig. 1. In Fig. 1, the VLDL/chylomicron remnant, LDL and HDL peaks are identified. Traces are representative for three animals for the control and two for the experimentals. The third experimental animal exhibited no change. Strikingly, the animal with the highest $LDLR_{KDEL}$ expression level, as determined by Western blot analysis, showed an about 50% reduction in plasma cholesterol levels (245.8 mg/dl before injection and 124.6 mg/dl after). Cholesterol levels showed little or no change in plasma from control animals.--

Page 8, paragraph beginning line 23, please substitute the following:

--The second trial using the $LDLR_{KDEL}$ vector *in vivo* was performed in mice which possessed a wild-type LDL receptor. In this trial the control selected was a plasmid encoding a protein that differs from the KDEL motif by a single amino acid substitution (Ile (140) to Asp). This variant, designated KDEL-ID, was predicted to be deficient in apoB binding and appeared from *in vitro* experimentation to be a suitable control. Mice were injected in a tail vein with 25 [m]μg of DNA coding for either $LDLR_{KDEL}$ or the KDEL-ID variant. Experiments were performed using the Trans-IT In Vivo protocol (Mirus Corporation) according to the manufacturer's instructions. Plasma was harvested approximately 48 hours after injection following a 4 hour fast. The recovered plasma was diluted 1:1 with PBS, filtered and lipoprotein particles were separated on a Sepharose 6 gel filtration FPLC column (Pharmacia). Cholesterol values for each fraction were determined enzymatically (Sigma). The data is shown in Fig. 2, which represents the mean values for three animals for each treatment, the error bars representing standard error of the mean. The VLDL/chylomicron remnant, LDL and HDL peaks are indicated. This data demonstrates a reproducible lowering of cholesterol levels by about 20%. This result is striking due to the quite low initial VLDL/LDL cholesterol levels in these mice. Additionally, these results may be understated. A mouse HDL particle (HDL-1) co-migrated with LDL and thus may partially mask the effect from the LDL_{KDEL} treatment.--

In the Claims

Please amend Claims 1, 5 and 9 as follows and withdraw Claims 13-16 without prejudice:

1. (Amended) A method for the lowering of serum cholesterol levels in [an individual] a mammal comprising the steps of

making a genetic construct comprising (1) a protein coding sequence encoding for the expression of a fusion protein, the fusion protein including a low density lipoprotein receptor which does not include the domain of the native protein associated with membrane binding and a localization domain which directs localization of the fusion protein to the interior of a cell in the [individual] mammal, and (2) a promoter effective in the cells of the [individual] mammal to express the protein coding sequence; and

delivering the genetic construct into the [individual] mammal such that the expression and production of the fusion protein in the mammal results in the lowering of serum cholesterol in the mammal.

5. (Amended) A method for the lowering of plasma triglyceride levels in [an individual] a mammal comprising the steps of

making a genetic construct comprising (1) a protein coding sequence encoding for the expression of a fusion protein, the fusion protein including a low density lipoprotein receptor which does not include the domain of the native protein associated with membrane binding and a localization domain which directs localization of the fusion protein to the interior of a cell in the [individual] mammal, and (2) a promoter effective in the cells of the [individual] mammal to express the protein coding sequence; and

delivering the genetic construct into the cells of the [individual] mammal such that the expression and production of the fusion protein in the mammal results in the lowering of plasma triglycerides in the mammal.

9. (Amended) A DNA construct comprising a promoter operably linked to a protein coding sequence, the protein coding sequence coding for the expression of a fusion protein comprising a low density lipoprotein receptor which does not include the domain of the native protein associated with membrane binding and a localization domain signaling for the transport of the fusion protein to the interior of a cell.